Agricultural Analytical Chemistry
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, Indiana 46140

DETERMINATION OF FLURIDONE[1] RESIDUE IN CROPS BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

AM-AA-CA-R052-AA-755

Principle

Fluridone is extracted from crops with methanol, and an aliquot of the extract is purified by liquid-liquid partitioning and alumina column chromatography. The purified extract is concentrated and then measured by high pressure liquid chromatography with a U.V. detector operated at a fixed wavelength (313 nm).

Chemicals and Reagents

1. Solvents
   a. Methanol, reagent grade
   b. Hexane, reagent-grade, redistilled
   c. Dichloromethane, reagent grade, redistilled
   d. Methanol, HPLC grade
   e. Water, HPLC grade
2. Solutions
   a. HPLC mobile phase--methanol (HPLC grade):water (HPLC grade), 60:40 (V/V), filtered and degassed.
   b. Hexane:dichloromethane (70:30, V/V)
   c. Sodium chloride, aqueous (5%, W/V)
3. Sodium sulfate, anhydrous, methanol washed
4. Alumina, Alcoa F-20, deactivated with 4% water (V/W), see Section H.

Equipment

1. Rotary vacuum evaporator (Rinco or equivalent) with water bath heated to approximately 35-45°C
2. Magnetic stirrer and stirring bar

---

1. 1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4-(1H)-pyridinone (coded EL-171)

3. Gyratory shaker (New Brunswick Model G33 or equivalent)

4. Chromatography columns—250 mm x 14 mm i.d., equipped with stopcock and 250 ml reservoir.

5. A high pressure liquid chromatograph consisting of the following components (or equivalent models):

   Waters Model 6000A solvent delivery system
   Waters Model 440 Absorbance Detector (fixed wavelength, 313 nm)
   Waters Model 710A Intelligent Sample Processor
   Houston Instruments Omni Scribe Strip Chart Recorder, 1-10mV

Procedure

A. Preparation of Standard Solutions

   1. Standard Solution A (fluridone, 1.0 mg/ml)—dissolve 100 mg of fluridone analytical standard in methanol (HPLC grade) in a 100-ml volumetric flask and dilute to volume.

   2. Standard Solution B (fluridone, 10.0 mcg/ml)--transfer a 1.0 ml aliquot of Standard Solution A to a 100-ml volumetric flask and dilute to volume with methanol:water (60:40).

   3. Standard Solution C (fluridone, 1.0 mcg/ml)—transfer a 10.0 ml aliquot of Standard Solution B to a 100-ml volumetric flask and dilute to volume with methanol:water (60:40).

   4. Standard Solution D (fluridone, 10.0 mcg/ml)--transfer 0.5 ml of Standard Solution A to a 50-ml volumetric flask and dilute to volume with hexane:dichloromethane (70:30).

B. Fortification of Recovery Samples

With each set of samples, prepare recovery samples in duplicate by fortifying two 25-g aliquots of an untreated crop sample with 2.5 ml of Standard Solution C. If insufficient control sample is available, prepare duplicate system recoveries by fortifying two system blanks (100 ml of methanol) with 2.5 ml of Standard Solution C. Also analyze an untreated control (if available) and a system blank with each set of samples.

C. Sample Extraction

1. Weigh 25 g of finely ground crop sample into a pint Mason jar. Add methanol (reagent-grade) to result in a total extraction volume of 100 ml with allowance for the moisture content of the crop. (Add a total of 200 ml for light-weight, bulky samples such as grass or straw.) Shake the sample for 30 minutes at 250 rpm on a gyratory shaker.

2. Transfer a 20-ml aliquot of the methanol extract to a graduated cylinder by pouring through a funnel containing folded filter paper. (Transfer a 40-ml aliquot if a 200-ml extraction volume was used.)

D. Liquid-Liquid Partition

1. Transfer the aliquot from step C-2 to a separatory funnel containing 20 ml of 5% sodium chloride solution. (Use 40 ml of sodium chloride solution if a 40-ml aliquot is collected.)

2. Rinse the graduated cylinder with 40 ml of hexane and transfer the rinse to the separatory funnel.

3. Shake the separatory funnel for approximately 20 seconds. Allow the phases to separate and drain the aqueous (lower) phase into a beaker. Discard the hexane (upper) phase and return the aqueous phase to the separatory funnel.

4. Repeat the extraction (step 3) with an additional 40-ml aliquot of hexane.

5. Extract the aqueous phase three times by shaking with three 40-ml aliquots of dichloromethane. Allow the phases to separate and drain the dichloromethane (lower) phase through a funnel containing sodium sulfate into a 250-ml evaporating flask. After the third extraction, rinse the sodium sulfate with 15-20 ml of dichloromethane.

6. Evaporate the dichloromethane just to dryness with a rotary vacuum evaporator and a 35-45°C water bath. Dissolve the residue in 5 ml of hexane:dichloromethane (70:30).

E. Alumina Column Purification

1. Prepare a column with standardized, deactivated alumina according to the procedure in Section H.

2. Add the sample extract from step D-6 to the column and drain to the top of the sodium sulfate. Discard the eluate.

3. Rinse the flask with 5 ml of hexane:dichloromethane (70:30) and add the rinse to the column. Drain to the top of the sodium sulfate and repeat with a second 5 ml rinse.

4. Wash the column with an additional 25 ml of hexane:dichloromethane (70:30) and discard the eluate.

5. Wash the column with 30 ml of dichloromethane and discard the eluate.

6. Add an additional 50 ml of dichloromethane and collect the eluate in a clean 125 ml evaporating flask.

NOTE: See Section H for standardizing the elution volume to be collected.

7. Evaporate the dichloromethane just to dryness with a rotary vacuum evaporator and a 35-45°C water bath.

8. Dissolve the residue in 4.0 ml of methanol:water (60:40). If the solution contains suspended particulate matter, filter the solution and transfer the filtrate to an HPLC sample vial.

F. HPLC Measurement

1. Measure the HPLC peak height response for fluridone using the instrumentation listed in the EQUIPMENT section and the following analytical parameters.

NOTE: The parameters listed below may be modified as needed to compensate for daily variations in instrument performance. The parameters used for the analysis should be recorded.

Column—μBondapak C_{18} or Lichrosorb RP-18 with a Co-Pell ODS guard column

Mobile phase—methanol:water (60:40)

Flow rate—0.8-1.3 ml/min

Injection volume—200 microliters

Attenuation—0.01 AUFS

Chart speed—0.167 cm/min

Direct Standard—Standard Solution C

2. During the sample analysis, periodically determine the HPLC peak height for Standard Solution C. Use the average peak height for calculating the results in Section G.

G. Calculations

$$1.\ \%\ \text{Recovery} = \frac{PH_{rec}}{PH_{std}} \times C \times V \times AF \times \frac{100\%}{\text{mcg fortified}}$$

where: PH_{rec} = peak height (cm) of recovery sample

PH_{std} = average peak height (cm) of standard

C = concentration (mcg/ml) of standard

V = final volume (ml), including dilutions

AF = aliquot factor (normally 5).

$$2.\ \text{parts-per-million (ppm)} = \frac{\frac{PH_{sa}}{PH_{std}} \times C \times V \times AF \times 100\%}{W \times \%\ \text{Recovery}}$$

where : PH_{sa} = peak height (cm) of sample

W = weight (g) of sample extracted

H. Deactivation and Standardization of Alumina

NOTE: Each batch of alumina must be deactivated and standardized prior to initial use.

1. Deactivation of Alumina

   a. Determine the loss on drying of the alumina as received by heating at 110°C for at least 4 hours. Add sufficient deionized water to result in a total moisture content of 4.00% (v/w).

   b. Tumble the alumina in a closed container for at least one hour.

2. Packing of Alumina Column

   a. Place a plug of glass wool in the bottom of a chromatography column. With the stopcock closed, add 15 ml of hexane:dichloromethane (70:30) followed by 10 ml (9.6 g) of alumina and 15 ml of hexane:dichloromethane (70:30).

   b. Push a plastic rod through the column packing, open the stopcock, and thoroughly stir the packing as it begins to settle. Remove the rod and rinse the column reservoir with 5 ml of hexane:dichloromethane (70:30).

   c. Add approximately 1 cm of sodium sulfate, rinse the reservoir with 5 ml of hexane:dichloromethane (70:30), and drain the solvent to the top of the sodium sulfate.

3. Standardization of Alumina

   a. Add 1.0 ml of Standard Solution D to the column, followed by 4 ml of hexane:dichloromethane (70:30) and drain the eluant to the top of the column.

   b. Add 5 ml of hexane:dichloromethane (70:30) and drain to the top of the column.

   c. Wash the column with 30 ml of hexane:dichloromethane (70:30) and discard the eluate.

   d. Elute the column with 120 ml of dichloromethane in 10 ml fractions and collect each fraction in separate 125 ml evaporating flasks.

e. Evaporate the fractions just to dryness and dissolve the residues in 5.0 ml of methanol:water (60:40).

f. Analyze each fraction by HPLC using the parameters listed in Section F. Compare the peak height response of fluridone in the column fractions with that of Standard Solution C to determine the elution pattern of fluridone, and adjust the volume of dichloromethane to be collected in Step E-6 accordingly.

DISCUSSION

The residue method is sensitive to 0.05 ppm and has resulted in recoveries (average ± standard deviation) of 87.3±16.8 percent for 24 untreated control crop samples fortified with 0.05 ppm and 0.10 ppm of fluridone. Recoveries obtained with four different crops are summarized in Table I. No background interference has been observed in the chromatograms of blank or control samples. Chromatograms demonstrating the recovery of fluridone from potatoes are contained in Figure 1.

S. O. West
R. O. Burger
811-4569

Ref. Notebook OK6

njm
10/15/81
I.D. 1278a

TABLE I. SUMMARY OF FLURIDONE RECOVERIES FROM CROPS

Crop	N	Recovery (avg. ± s.d.) 0.05 ppm	0.10 ppm
grapes	3	96.0 ± 16.0	86.7 ± 6.1
potatoes	3	101.1 ± 8.5	78.6 ± 12.1
grass	3	80.2 ± 21.8	59.2 ± 3.0
wheat grain	3	98.4 ± 3.1	98.4 ± 10.9

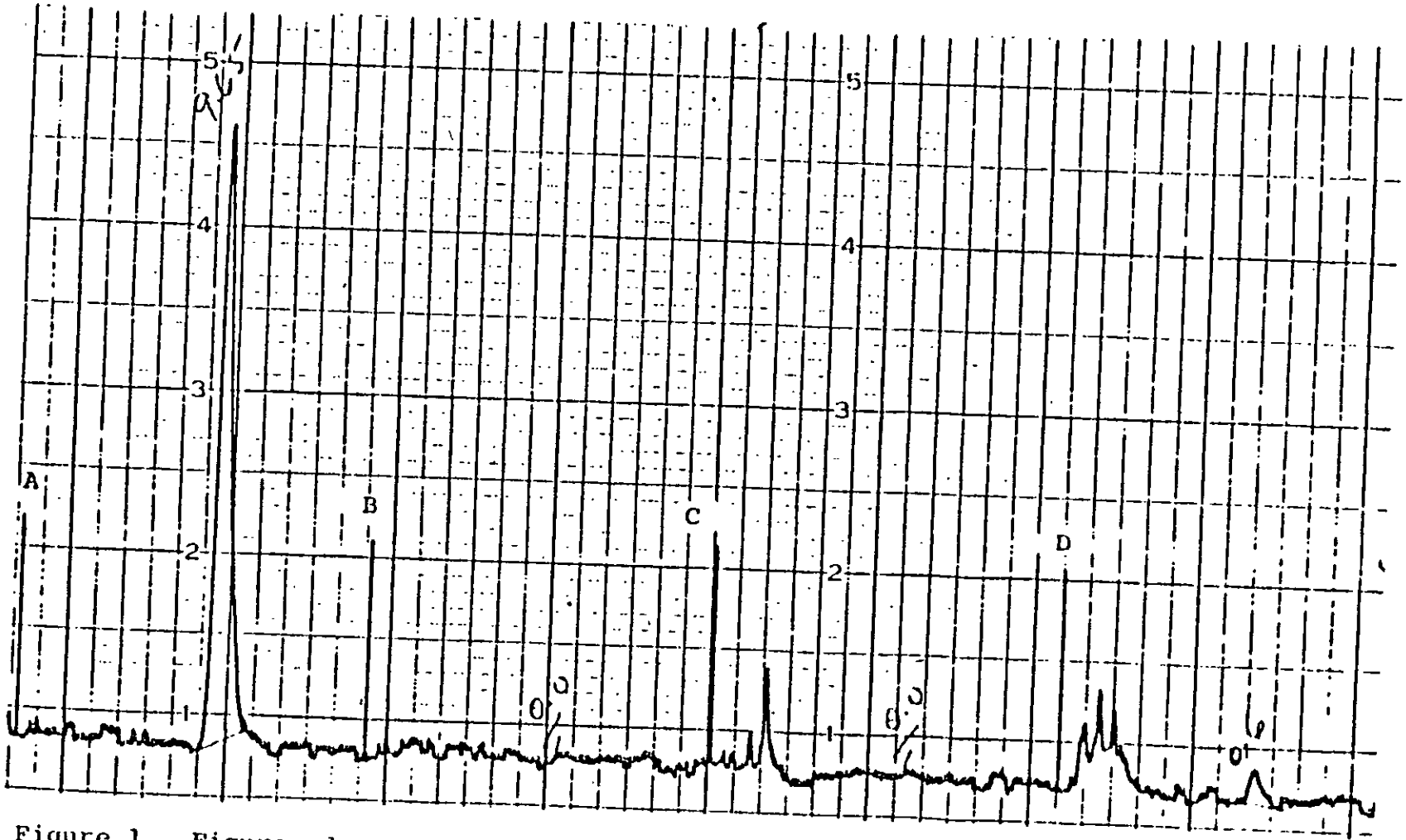

Figure 1. Figures demonstrating the recovery of fluridone from potatoes: (A) fluridone standard, 200 ng; (B) system blank; (C) untreated control potatoes; (D) untreated control potatoes fortified with 0.05 ppm of fluridone, equivalent to a 101% recovery.